



**For the detection of:** *Salmonella*, *Citrobacter*, *Proteus*, *Edwardsiella*, *Klebsiella* (some spp.)

**Media:** Pathoscreen™ Medium

**Sample Type:** drinking water, surface water, and recreational water

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## Detecting Bacteria and Pathogens

Many serious diseases, such as typhoid fever and dysentery, can be traced directly to pathogenic microorganisms in polluted water. These disease-producing organisms are discharged in fecal wastes and are difficult to detect in water supplies. People may come in contact with these pathogens in drinking water or in recreational waters such as swimming pools, rivers, streams, and bathing beaches.

Testing directly for bacterial pathogens is impractical for many reasons, not the least of which is the need for lengthy and involved test procedures. It has become customary to use *indicator organisms* instead. These are bacteria, usually not pathogenic, that are present when pathogens are present and absent when pathogens are absent. Indicator organisms are usually of fecal origin as well.

No one organism or group of organisms satisfies all of the criteria for an indicator. For example, in temperate climates total coliform bacteria are commonly used as indicator organisms in potable water supplies. In many tropical climates, however, indigenous *Escherichia coli* (*E. coli*) are present in pristine water sources where no fecal contamination exists; yet they will produce positive results in total coliform tests. In such cases, other bacteria, known to be associated with fecal contamination, may be used as indicator organisms in place of the coliforms. The hydrogen sulfide-producing bacteria have been shown to be associated with the presence of fecal contamination and total coliform bacteria,\* and they may be used as an indicator organism in place of coliforms.

Hach's PathoScreen™ Medium\*\* detects the presence of hydrogen sulfide-producing bacteria including *Salmonella*, *Citrobacter*, *Proteus*, *Edwardsiella*, and some species of *Klebsiella*. The sterilized powder medium is easy to use and produces easy-to-interpret results. This reliable, inexpensive medium is well suited for monitoring drinking water systems in developing tropical countries, in remote field locations, and in disaster or emergency situations.

## Convenient Packaging

PathoScreen Medium is dehydrated, sterilized, and packaged in powder pillows. Powder pillows are available for both Presence/Absence (P/A) and Most Probable Number (MPN) testing. Each powder pillow contains enough medium for one test. The medium is shipped with a Certificate of Analysis and has an expiration date printed on the label.

For P/A testing, add one P/A powder pillow to a 100-mL sample. For MPN testing, add one MPN Pillow to a 20-mL sample. For MPN testing, you will need to inoculate a set of five tubes.

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\* Kromoredjo, P.; Fujioka, R. S. Evaluating three simple methods to assess the microbial quality of drinking water in Indonesia. *Environmental Toxicology and Water Quality: An International Journal* **1991**, 6:259–270.

Manja, K. S.; Maurya, M. S.; Rao, K. M. A simple field test for the detection of fecal pollution in drinking water. *Bulletin of the World Health Organization* **1982**, 60:797–801.

\*\* PathoScreen™ is a Hach Company trademark.

# Bacteria: Hydrogen Sulfide Producing, continued

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## Specifications

**Incubation:** 24 to 48 hours between 25 to 35 °C (30 °C is optimal.)

**Sensitivity:** CFU/100 mL

**Selectivity:** Hydrogen sulfide-producing bacteria, including *Salmonella*, *Citrobacter*, *Proteus*, *Edwardsiella*, and some species of *Klebsiella*

## Technique is Important

Good laboratory technique is essential when accuracy is important, particularly in microbiological laboratory procedures. Care in sample collection and preservation, a clean laboratory or work surface, proper sterilization and inoculation practices, and close temperature control help assure reliable results.

## Preparing Sample Containers

Care must be taken to prevent contamination when conducting bacterial tests. All materials used for containing or transferring samples must be sterile. To collect samples, use any of the following: sterilized plastic bags, sterilized disposable bottles, autoclavable glass bottles, or autoclavable plastic bottles.

**Note:** *Dechlorinating reagent should be used with potable or chlorinated water samples. It is not necessary for unchlorinated or nonpotable water samples. However, dechlorinating reagent will not interfere with unchlorinated samples so, for simplicity, plastic bags containing dechlorinating reagent may be used for all samples.*

**Sterilized plastic bags or disposable bottles:** Pre-sterilized plastic bags and bottles are available with or without dechlorinating agent. The bottles are available with a 100-mL fill-to line.

**Autoclavable glass or plastic bottles:** Glass or plastic bottles (125-mL size) may be used instead of sterilized plastic bags or disposable bottles. These containers should be prepared as follows:

1. Wash in hot water and detergent.
2. Thoroughly rinse with hot tap water, followed by a distilled water rinse to make sure that all detergent is removed.
3. If dechlorinating agent is needed (for chlorinated, potable water), add the contents of one Dechlorinating Reagent Powder Pillow for each 125-mL of container volume. (A 250-mL sample container will require two powder pillows.)
4. Steam sterilize glass and autoclavable plastic containers at 121 °C for 15 minutes. Glass sample containers may be sterilized by hot air at 170 °C for one hour.
5. Store sterile containers, tightly capped, in a clean environment until needed.

## Collecting and Preserving Samples

### General Guidelines

Use proper sampling procedures to insure that seasonal variances are detected and that results are representative of the sample source. Collect a sufficient volume of sample (usually 100 mL) for the guidelines to be met. The World Health Organization guidelines prescribe 200 mL per sample, while *Standard Methods for the Examination of Water and Wastewater* prescribes 100 mL per sample. Maintain at least 2.5 cm (approximately 1 inch) of air space to allow adequate space for mixing the sample prior to analysis.

Avoid sample contamination during collection. Carefully open each sample container just prior to collection, and close immediately following collection. Do not lay the lid or cap down and avoid touching the mouth or the inside of the container. Do not rinse the container.

No dechlorination is necessary if the sample is added directly to the medium on site. Otherwise, samples should be treated to destroy chlorine residual and immediately transported for analysis after collection. Sodium thiosulfate, sterilized within the collection container, is commonly used to destroy chlorine residual.

Properly labeled samples should arrive at the testing facility within 24 hours after collection. In warm climates, the samples must be packed in a freezing mixture to maintain the sample temperature between 4 and 10 °C. Failure to properly collect and transport samples will cause inaccurate results.

### Collecting from faucets, spigots, hydrants or pumps:

Collect representative samples by allowing the water to run from a faucet, spigot, hydrant, or pump at a moderate rate, without splashing, for two to three minutes before sampling. Do not adjust the rate of flow while the sample is being collected. Valves, spigots, and faucets that swivel or leak should be avoided. Remove aerators or screens attached to valves, spigots, and faucets before collecting samples.

### Collecting from rivers, lakes and reservoirs:

When sampling a river, lake or reservoir, fill the sample container below the water surface. Do not sample near the edge or bank. Remove the cap, grasp the sample container near the bottom, and plunge the container, mouth down, into the water. (This technique excludes any surface scum.) Fill the container by positioning the mouth into the current, or, in nonflowing water, by slightly tilting the bottle and allowing it to fill slowly.

## Disposing of Completed Tests

Active bacterial cultures grown during incubation must be disposed of safely. This may be accomplished in one of two ways.

**Bleach.** Used test containers may be sterilized by using a 10% bleach solution. Add approximately 12 mL of bleach to each test container. Allow 10 to 15 minutes contact time with the bleach. Pour the liquid down the drain, then dispose of the test containers in the normal garbage.

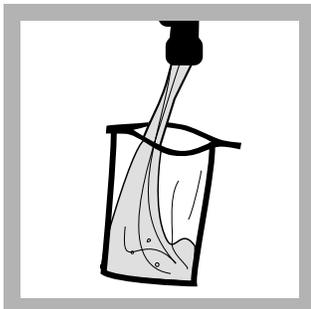
**Autoclave.** Place used test containers into a contaminated items bag or a biohazard bag and seal tightly to prevent their leaking into the autoclave. Autoclave at 121 °C for 15 minutes at 15 pounds pressure. Place the bag of sterilized test containers in a separate garbage bag and tie tightly; dispose of with the normal garbage.

# Bacteria: Hydrogen Sulfide Producing, continued

## Using PathoScreen Medium P/A Pillows Method 10032



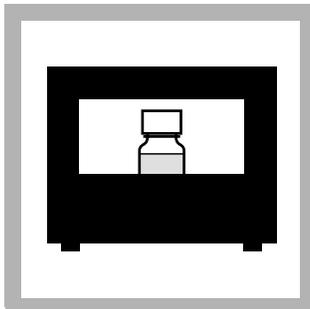
**1.** Wash your hands thoroughly with soap and water.



**2.** Collect 100 mL of sample in a sterile sample container. (See *Collecting and Preserving Samples* for details.)



**3.** Swab the end of the PathoScreen Medium P/A Pillow with alcohol and aseptically cut it open with clippers. Add pillow contents to the 100 mL sample.



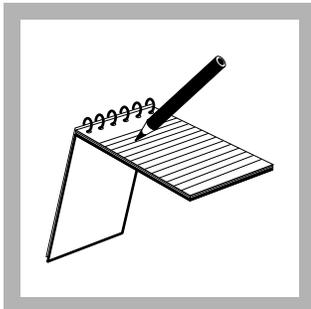
**4.** Place the bottle in a location with a constant temperature between 25 and 35 °C for 24 to 48 hours.

**Note:** If an incubator is available, incubate the sample at  $30 \pm 0.5$  °C for 24 to 48 hours.

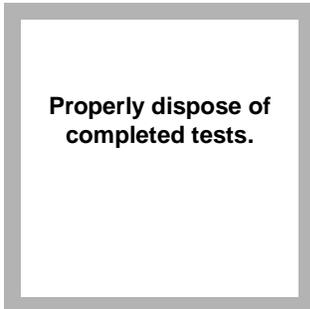


**5.** Note the reaction after 24 hours of incubation.

**Note:** If the temperature varies significantly, incubation may be extended an additional day.



**6.** Record results. (See *Interpreting Results.*)



**7.** Dispose of all completed tests appropriately. (See *Disposing of Completed Tests.*)

# Bacteria: Hydrogen Sulfide Producing, continued

## Interpreting Results

Hydrogen sulfide producing bacteria			
Test Results	Positive	Negative	Follow-up
Color changes from yellow to black	X		
Black precipitate forms	X		
No color change		X	Incubate additional 12 to 24 hours and reevaluate. If there is no color change, record as negative.

## Conducting MPN Tests with PathoScreen Medium

The MPN method can be used for drinking water, as well as marine and fresh recreational waters, swimming pools, lakes, shellfish-growing waters, heavily polluted waters, and wastewater. For water that is heavily contaminated, use the multiple tube decimal dilution procedure.

### Sample Size and Dilution — Nonpotable Water

Testing nonpotable water samples may require dilution of the original sample, based on the density of organisms. For example, when examining swimming pool water, inoculate five tubes with 20 mL of a 1:10 dilution of sample. *Table 1* specifies typical dilutions used for various sample types.

#### Preparing Dilution Water

Sterile, buffered dilution water is available in 99-mL bottles or it can be prepared by one of the following methods:

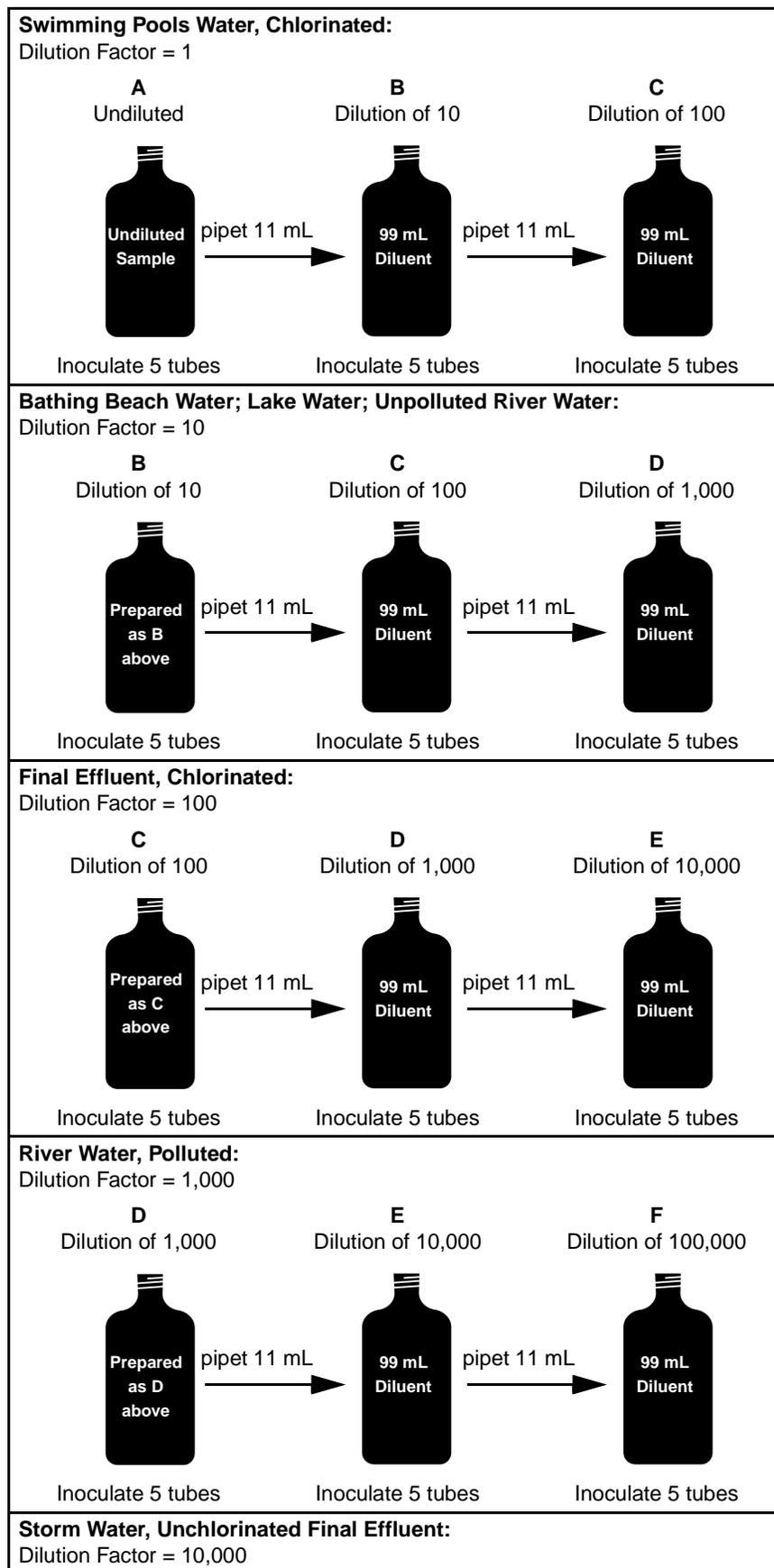
1. Add the contents of one Peptone Powder Pillow to one liter of distilled or deionized water. Dispense in amounts to yield  $99 \pm 2$  mL after autoclaving. Sterilize in an autoclave for 15 minutes.
2. Add the contents of one pH adjusted Potassium Dihydrogen Phosphate Pillow and one Magnesium Chloride Pillow to one liter of distilled or deionized water. Dispense in amounts to yield  $99 \pm 2$  mL after autoclaving. Sterilize in an autoclave for 15 minutes.

#### Dilution Technique

1. Wash hands.
2. Open a bottle of sterile Buffered Dilution Water.
3. Shake the sample collection container vigorously, approximately 25 times.
4. Using a sterile transfer pipet, pipet the required amount of sample into the sterile Buffered Dilution Water.
5. Recap the Buffered Dilution Water bottle and shake vigorously 25 times.
6. If more dilutions are needed, repeat *steps 4* and *5* using clean, sterile pipets and additional bottles of sterile Buffered Dilution Water.

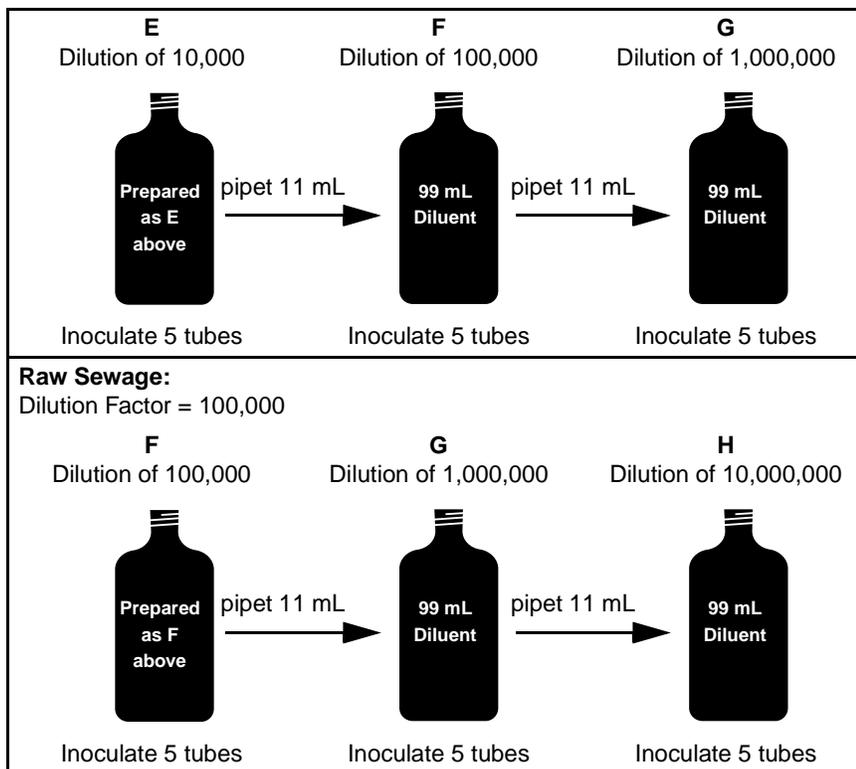
# Bacteria: Hydrogen Sulfide Producing, continued

**Table 1 Typical sample decimal dilutions for nonpotable water (MPN testing)**



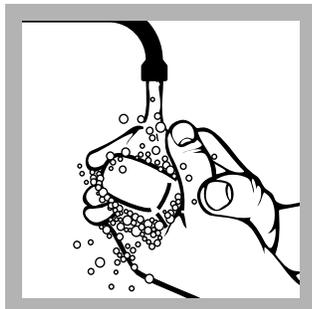
# Bacteria: Hydrogen Sulfide Producing, continued

**Table 1 Typical sample decimal dilutions for nonpotable water (MPN testing) (Continued)**



# Bacteria: Hydrogen Sulfide Producing, continued

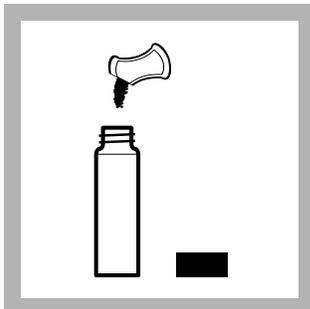
## Using PathoScreen Medium MPN Pillows Method 10032



**1.** Wash your hands thoroughly with soap and water.



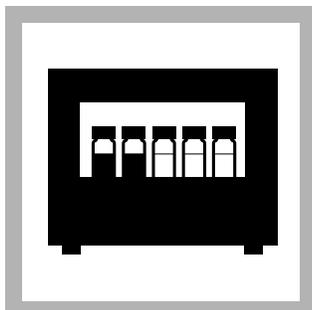
**2.** Remove the caps from five sterile tubes one at a time and pipet 20 mL of sample into each of the tubes with a sterile pipet. Use aseptic technique to avoid contaminating the tubes or the caps.



**3.** Swab the end of a PathoScreen Medium MPN Pillow with alcohol and aseptically cut it open with clippers. Add pillow contents to the 20 mL sample.



**4.** Cap each tube immediately. Invert the tubes a few times to thoroughly mix the sample with the medium.



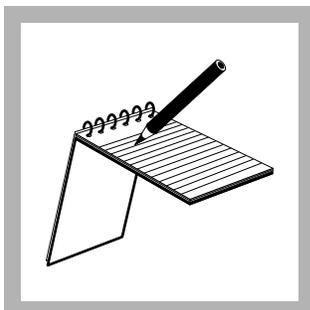
**5.** Place the bottle in a location with a constant temperature between 25 and 35 °C for 24 to 48 hours.

**Note:** If an incubator is available, incubate the sample at  $30 \pm 0.5$  °C for 24 to 48 hours.



**6.** Note the reaction after 24 hours of incubation. (See *Interpreting Results.*)

**Note:** If the temperature varies significantly, continue to incubate negative tubes for an additional day.



**7.** Record results. (See *Table 2.*)



**8.** Dispose of all completed tests appropriately. (See *Disposing of Completed Tests.*)

# Bacteria: Hydrogen Sulfide Producing, continued

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## Interpreting Results

Hydrogen sulfide producing bacteria			
Test Results	Positive	Negative	Follow-up
Color changes from yellow to black	X		
Black precipitate forms	X		
No color change		X	Incubate additional 12 to 24 hours and re-evaluate. If there is no color change, record as negative.

Using statistical methods it is possible to estimate the number of organisms from any combination of positive and negative test results. The MPN values in *Table 2* are based on 20 mL of undiluted sample in each of five tubes. If the sample is diluted, multiply the result by the dilution factor.

**Example 1:** Five tubes of undiluted sample are inoculated. Positive results are obtained from three of the five tubes. The result obtained from *Table 2* is 4.6.

**Example 2:** A river water sample is collected and diluted. A dilution factor of 10,000 is prepared and five tubes are inoculated. Positive results are obtained from two of the five tubes. The result obtained from two of the five tubes. The result obtained from *Table 2* is 2.6. This result is multiplied by 10,000, and a result of 26,000 is recorded.

**Table 2 Five-tube MPN values for undiluted, 20-mL samples (95% confidence limits)**

Positive Tubes	MPN/100 mL
0	<1.1
1	1.1
2	2.6
3	4.6
4	8.0
5	>8.0

# Bacteria: Hydrogen Sulfide Producing, continued

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## Medium, Reagents and Apparatus

### Medium

Description	Unit	Cat. No.
PathoScreen™ Medium, P/A Pillows, 100-mL sample .....	50/pkg .....	26106-96
PathoScreen™ Medium, MPN Pillows, 20-mL sample.....	50/pkg .....	26107-96

### For Dilution Water

Bottle, polysulfone, autoclavable (for preparing buffered dilution water) .....	12/pkg .....	22453-00
Buffered Dilution Water, sterile, 99-mL* .....	25/pkg .....	14305-98
Dechlorinating Reagent Powder Pillows.....	100/pkg .....	14363-69
Magnesium Chloride and Potassium Dihydrogen Phosphate Powder Pillows .....	25 of each .....	21431-66
Peptone Powder Pillows, 1-g.....	30/pkg .....	21429-64
Pipet, sterile, disposable, 11-mL .....	25/pkg .....	2097-98
Pipet, sterile, disposable, individually wrapped, 10-mL .....	50/pkg .....	20926-28
Pipet, sterile, disposable, 10-mL .....	50/pkg .....	20928-28
Pipet Aid with:		
110 Vac Recharger (UL, CSA approved) and 4 replacement filters .....	each .....	25517-01
220 Vac Recharger (UL, CSA approved) and 4 replacement filters .....	each .....	25517-02

### Apparatus

Alcohol Burner, 100-mL .....	each .....	20877-42
Autoclave, Automatic:		
120 Vac .....	each .....	24630-00
240 Vac .....	each .....	24630-02
Clippers, large .....	each .....	20658-00
Contaminated Items Bags.....	200/pkg .....	24633-00
Germicidal Cloth .....	50/pkg .....	24632-00
Incubator, Culture:		
120 Vac .....	each .....	26192-00
220 Vac .....	each .....	26192-02
MPN Vials .....	10/pkg .....	14970-54
Rack for coliform tubes.....	each .....	24979-03

### Sampling Containers

Sampling Bags, Whirl-Pak with dechlorinating agent, 170-mL .....	100/pkg .....	20753-33
Sampling Bottles, autoclavable .....	6/pkg .....	23243-33
Sampling Bottles, autoclavable .....	48/pkg .....	23243-73
Sampling Bottles, sterilized, 100-mL fill-to line.....	12/pkg .....	24950-12
Sampling Bottles, sterilized, 100-mL fill-to line.....	50/pkg .....	24950-50
Sampling Bottles, sterilized, 100-mL fill-to line with dechlorinating agent.....	12/pkg .....	25991-12
Sampling Bottles, sterilized, 100-mL fill-to line with dechlorinating agent.....	50/pkg .....	25991-50

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\* Buffered Dilution Water is prepared with magnesium chloride and potassium dihydrogen phosphate.





**FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:**

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

On the Worldwide Web – [www.hach.com](http://www.hach.com); E-mail – [techhelp@hach.com](mailto:techhelp@hach.com)

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